MECHANICAL PROPERTIES AND MASS DIFFUSION PROPERTIES IN FIBRIN HYDROGELS ARE UNDER DISTINCT CONTROL

Introduction

Cell fate is a function of mechanical and mass transport properties of its milieu. For example cell migration speed is influenced by substrate stiffness, while mass transport of distinct molecular agents in the cell-milieu controls distinct cell signalling pathways. Thus, tissue engineering applications find an obvious benefit in separate control of mechanical and mass transport. In this study, we demonstrate that mechanical response and microstructure of fibrin hydrogels can be modified separately from mass transport properties. It is expected that separate control in fibrin hydrogels will lead to them playing an important role in tissue engineering [Drury, 2003].

Methods

Fibrin hydrogels with concentrations of fibrinogen (5, 10 and 20 mg/ml), thrombin (0.02 and 0.2 U/mg fibrinogen) and factor XIII (0.02 and 2 U/mg fibrinogen) were tested using shear rheology for the estimation of storage shear modulus (G′). Separate samples were imaged by confocal microscopy, and a fiber extraction algorithm [Stein, 2008] was used to analyse gel microstructure. FRAP (Fluorescence Recovery after Photobleaching) was used for measuring the diffusion coefficient of 10 and 40 kDa dextran solutes through the hydrogels.

Results

Fibrinogen and factor XIII concentrations were found to positively influence G′, while thrombin has a strong negative influence (table 1). These trends were valid over a range of physiological frequencies (0.01-100 rad/s) and strains (0.01%-10%). Image analysis of the gel microstructure showed that at low levels of thrombin and factor XIII, increase in fibrinogen concentration leads to increase in number of fibers per unit of volume (from 8.5 to 23.1 $x10^7$ fibers/mm^3 at 5 and 20 mg/ml fibrinogen respectively). Increase in thrombin and factor XIII causes a decrease in fiber radius (from 130 nm at low concentrations of both enzymes to 117.5 nm when thrombin is increased and 116.2 nm when factor XIII is increased, at a density of 10 mg/ml) and a rise in fiber length. The concentration of the two enzymes positively affects the number of fibers per unit of volume, especially for the highest concentration of fibrinogen (from 23.1 at low concentrations of the two enzymes, to 33.7 and 28.6 $x10^7$ fibers/mm^3, when thrombin and factor XIII are increased, respectively). Finally, diffusivity measurements showed that increasing fibrinogen concentration leads to lower diffusivity values (from 125.7 and 57.9 $\mu m^2/s$ at 5 mg/ml to 113.4 and 53.4 $\mu m^2/s$ at 20 mg/ml for 10 and 40 kDa dextran, respectively), but the effects of thrombin and factor XIII do not have a clear trend.

Discussion

Based on the presented data it is evident that variations in the composition of fibrin result in moderate changes in terms of diffusivity, but in significant changes in the fibrillar structure and order of magnitude changes in mechanical properties of the hydrogels. This selective control makes fibrin hydrogels a promising candidate in tissue engineering applications.

Gel Composition	G′ [Pa]
F (5 mg/ml), T (0.02 U/mg), f13 (0.02 U/mg)	237
F (5 mg/ml), T (0.2 U/mg), f13 (2 U/mg)	67
F (20 mg/ml), T (0.02 U/mg), f13 (2 U/mg)	1570
F (20 mg/ml), T (0.2 U/mg), f13 (0.02 U/mg)	263

Table 1: Storage shear modulus (at 1% strain and 1 rad/s frequency) is controlled strongly by fibrinogen (F), thrombin (T) and factorXIII (f13) concentrations.

References

Drury *et al*, Biomaterials, 24:4337–51, 2003.
Steiner *et al*, J Microsc, 232:463–75, 2008.